UNCLASSIFIED

AD NUMBER

ADB259028

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Proprietary Info.; Oct 99. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott St., Fort Detrick, MD 21702-5012.

AUTHORITY

USAMRMC ltr, 1 Jun 2001.

THIS PAGE IS UNCLASSIFIED

AD_______________

Award Number: DAMD17-94-J-4251

TITLE: Biological Markers of Environmental Carcinogens in Breast Cancer

PRINCIPAL INVESTIGATOR: Frederica P. Perera, Ph.D.

CONTRACTING ORGANIZATION: Columbia University
New York, New York 10032

REPORT DATE: October 1999

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Oct 99). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-94-J-4251
Organization: Columbia University
Location of Limited Rights Data (Pages):

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

09/22/0

REPORT DOCUMENTATION PAGE

OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1999	Final (1-Oct-94 - 30-Sep-99)

4. TITLE AND SUBTITLE
Biological Markers of Environmental Carcinogens in Breast Cancer

5. FUNDING NUMBERS
DAMD17-94-J-4251

6. AUTHOR(S)
Frederica P. Perera, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Columbia University
New York, New York 10032

E-MAIL:
fpp1@columbia.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, Oct 99). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

We have completed a hospital based molecular epidemiologic study of breast cancer that included 119 cases, 108 benign breast disease (BBD) controls and 141 healthy controls. Blood samples were collected prior to surgery from surgical enrollees and from the healthy controls during routine gynecologic exams. Tumor and normal tissue from cases and benign tissue from BBD controls was collected from the pathology blocks. The primary biomarker analyses included aromatic-, heterocyclic amine-, and smoking related-DNA adducts in white blood cells, PAH-DNA adducts and p53 mutations in breast tissue. Another goal of the study was to create a repository of tissue and blood samples linked to pathology and questionnaire data to support future studies. These samples were used for complimentary analyses of xenobiotic metabolizing genes (GSTM1, NAT2 and CYP1A1) and pilot studies of oncoproteins (cyclin D1 and ras p21) and organochlorines (DDE, DDT and PCBs) in blood. The study generated data that support the hypothesis that environmental exposures to PAH and gene-environment interactions play a role in breast cancer etiology. It also generated pilot data indicating that oncoprotein markers can be detected in blood samples and are predictive of breast cancer status. These findings suggest new approaches to breast cancer prevention and to its early detection and prevention.

14. SUBJECT TERMS
Breast Cancer, Environmental Carcinogens, Carcinogen DNA Adducts, Prevention, Molecular Epidemiology, p53 Mutation, Humans, Anatomical Samples

15. NUMBER OF PAGES
28

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

N/A Where copyrighted material is quoted, permission has been obtained to use such material.

N/A Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

N/A Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

JPS
X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

11/1/99

PI - Signature Date

Table of Contents

	Page
Standard Form (SF) 298	2
Foreword	3
Table of Contents	4
Introduction	5
Body	5
Key Research Accomplishments	18
Reportable Outcomes	19
Conclusions	22
References	23
Bibliography of Published Papers and Abstracts	25
Personnel	27

INTRODUCTION

Breast cancer afflicts one in nine women by the age of 85 and is the second leading cause of cancer death among American women. In the U.S., the disease currently afflicts one out of nine women by the age of 85, with an estimated 175,000 new cases and 43,300 deaths in 1999 [1]. It is likely that environmental factors (including exposures related to lifestyle, occupation and ambient pollution) are contributors, particularly in high-risk areas such as the northeastern United States. Environmental contaminants such as the polycyclic aromatic hydrocarbons (PAH), heterocyclic amines (HA), cigarette smoke constituents and organochlorine residues are suspected mammary carcinogens of concern [2-4]. To test the hypothesis that exposure to these compounds are a risk factor for developing breast cancer a hospital based molecular epidemiologic case-control study was initiated at the Columbia Presbyterian Medical Center (CPMC). The study enrolled women referred for breast surgery; and pre-surgical blood samples were collected. Women with a final diagnosis of breast cancer were classified as cases and women with benign diagnoses without atypia were classified as benign breast disease (BBD) controls. Tumor tissue and normal tissue were retrieved from pathology specimens from cases; and benign tissue was retrieved from pathology specimens from BBD controls. A second non-surgical healthy control group was enrolled from among women coming to gynecological (GYN) practices for routine checkups. Women in the healthy control group donated a blood sample. All of the women took part in structured interview that gathered information on known breast cancer risk factors, environmental exposures, diet and cigarette smoking.

The original goals of the study were to measure heterocyclic amine (HA)-, PAH-, and smoking related-DNA adducts in mononuclear white blood cells, PAH-DNA adducts in breast tissue and p53 mutations in breast tumor tissue. No major changes to these goals occurred during the course of the study. However, after assaying 150 blood samples for HA-DNA adducts it was apparent that the women in the study were not receiving high enough exposures to heterocyclic amines to have detectable levels of HA-DNA adducts. As described in prior progress reports, this assay was halted and funds were used to partially support the analyses of polymorphisms in xenobiotic metabolizing genes (GSTM1, NAT2 and CYP1A1). In addition, data and blood samples collected in this study supported several innovative pilot studies of oncoproteins and organochlorines.

BODY OF THE FINAL REPORT

1. Patient Recruitment, Sample and Data Collection

a. Patient Enrollment

Patient enrollment was completed in June of year four. Active patient surveillance programs were conducted in the breast clinic and the offices of collaborating breast surgeons, Drs. Estabrook and Schnabel. Cases and BBD controls were enrolled by two interviewers under the direction of these two surgeons and their staff. All patients undergoing breast surgery with these doctors were evaluated as potential subjects. Eligible patients were identified and enrolled after the physician recommended surgery, but before surgery was performed. The study objectives and the patient's role in the study were explained to each of the prospective subjects and interested patients signed a consent form that met DOD and CPMC institutional requirements. Following enrollment, the patient was interviewed and a blood sample was drawn. Blood samples were drawn prior to surgery to prevent confounding of biomarker data by exposures to anesthesia, chemotherapy, hormone therapy, biologic changes associated with the healing process, or post-surgical changes in diet.

Healthy control subjects were enrolled through the GYN clinic and the private practices of Drs. Kelley and Levine and their staff. Dr Kelley and Levine's GYN practices are in the same building as the CPMC Breast Service; and these doctors refer their patients to Drs. Estabrook and Schnabel for breast health care. Further, data on birth date and residential zip code were analyzed from a random sample of each physician's patients and were found to be similar across the physician's practices. Women were approached during routine GYN check ups with Drs. Levine and Kelley and were enrolled into the study. These women signed a consent form, donated blood samples, and took part in the structured interview. Healthy control patients were frequency matched on age and ethnicity to cases

As a result of these surveillance programs, patient enrollment occurred at a faster pace than originally anticipated, with a total of 417 patients enrolled (see Table 1). This over-sampling assured us that we would have complete data and samples (questionnaire and medical record data and tissue and blood samples) for a total of 300 subjects (100 cases, 100 BBD controls and 100 healthy controls).

Table 1. Patient Enrollment

CATEGORY	ENROLLED
Total Enrolled Patients	417
Cases	119
BBD Controls	108
Healthy Controls	141
Other*	46
Unknown**	3

* includes: benign breast disease with atypia, lobular carcinoma in situ, and rare cancers (e.g. Cystosarcoma phylloides).

** Complete pathology reports are still unavailable on for three subjects.

A total of 120 patients were asked to take part in the study but refused to enter it. Of these, 88 were prospective surgical enrollees and 32 were prospective healthy controls. Among surgical patients the response rate was 76%; and among healthy subjects the response rate was 82% for an over all response rate of 78%. Non-responding surgical patients were on average older than the respondents, 60.8 years versus 53.1 years old, but had a similar ethnic distribution and the same prevalence of clinic versus private patients. Non-responding healthy controls were also older than responding healthy controls and were less likely to be Caucasian (29% of non-respondents were Caucasian, while 62% of respondents were Caucasian, p=0.001). They were also less likely to be private patients (55% of non-respondents were private patients, while 79% of the respondents were private patients, p=0.005).

b. Questionnaire and Pathology Data

Each of the patients took part in a structured interview that covered demographic variables, reproductive and health histories, diet, residential history, smoking, alcohol consumption, occupational history and environmental exposures. Interviews were conducted prior to surgery during the pre-operative testing procedures. Data from the questionnaires were abstracted into a computer spreadsheet as soon as the interview was completed. The spread sheet is in MS Exel format for simple importation into the SPSS and other statistics package. Pathology reports and data on receptor, proliferative and clinical markers analyzed by the pathology department (estrogen/progesterone receptor status, *erbB-2*, DNA index, G0-G1, S, and G2-M cell cycle status) have also been collected and abstracted into the same spreadsheet as the questionnaire data. Additionally, information on stage and tumor size has been collected from the CPMC Tumor Registry.

The basic breast cancer risk factor model included variables for age (as a continuous variable), ethnicity (Caucasian or non-Caucasian), history of breast feeding (yes or no), regular current alcohol consumption (yes or no), family history of breast cancer (yes or no), age at first birth (as a mean centered continuous variable), parity (parous or non-parous), age at menarche (less than age 13 or greater than equal to age 13). Odds ratios and 95% confidence intervals for these risk factors comparing cases to BBD controls and also cases to healthy controls are presented in Table 2. Comparing cases to BBD controls, age, ethnicity and alcohol consumption were significantly associated with case-BBD control status. In the comparison of cases to healthy controls age was significantly associated with case-control status. These findings are consistent with the literature. That more of the established risk factors were not significantly associated with case-control status is not surprising considering that this study was not powered to analyze questionnaire data on reproductive factors. These breast cancer risk factors were included in multivariate models that controlled for known breast cancer risk factors.

Table 2. Associations[1] Between Breast Cancer Risk Factors and Case-Control Status

	Cases Vs. BBD Controls OR (95% CI)	Cases Vs. Healthy Controls OR (95% CI)
Age (per year)	1.07 (1.04-1.11)	1.02 (1.00-1.05)
Ethnicity		
Non-Caucasian	1	1
Caucasian	2.04 (1.02-4.06)	1.50 (0.82-2.75)
Breast Feeding History		
Never	1	1
Ever	1.01 (0.50-2.02)	0.78 (0.44-1.40)
Regular Current Alcohol		
No	1	1
Yes	1.95 (1.05-3.62)	1.66 (0.97-2.86)
Family History of Breast Cancer		
No	1	1
Yes	0.87 (0.40-1.89)	1.35 (0.68-2.71)
Age at First Birth (per year)	1.04 (0.96-1.12)	0.98 (0.93-1.04)
Parity		
Parous	1	1
Non-parous	0.47 (0.19-1.14)	0.66 (0.30-1.47)
Menarche		
< age 13	1	1
≥ age 13	1.05 (0.57-1.92)	1.06 (0.63-1.79)

1 All odds ratios adjusted for all other risk factors in the table

Questionnaire data on PAH related exposures were analyzed for case-control associations. Information on active smoking including current smoking status, smoking as a teen, duration of smoking and packyears of smoking was considered. Information on environmental tobacco smoke exposure was also considered including current exposure, duration with which the subject lived with a smoker, exposure as a teen, and the number of family members who smoked when the subject was a teen. Lastly dietary practices associated with PAH exposure were also assessed, including consumption of broiled meats and the propensity to cook food so that it was blackened or charred. After control for known breast cancer risk factors, none of the questionnaire derived variables were associated with breast cancer case-control status and all of the odds ratios were close to unity

c. Biological Specimen Collection and Storage

Blood samples were collected from subjects and separated into total white blood cell, red blood cell, mononuclear white blood cells, and plasma. In addition to preserving the blood samples for the assays funded under this proposal, our design called for storing of aliquots for future research. Sample aliquots have been processed and stored in anticipation of future analyses of; 1) organochlorines, 2) plasma vitamin C and E, retinoids and carotinoids, 3) hemoglobin adducts, 4) plasma *erbB-2* extra-cellular domain, 5) plasma *ras* levels, 6) plasma p53 levels, 7) plasma EGFR levels, 8) plasma cyclin D1, 9) biomarkers of oxidative damage, and 10) metabolic genotype (NAT2, GSTM1, CYP1A1). This has created a sample bank that will allow future research to be conducted in an efficient and economical manner. Under separate funding preliminary analyses of several of these markers have been conducted to support spin-off studies (see Additional Studies of Genetic Susceptibility and Oncoproteins).

Due to the small size of many of the lesions, frozen tissue is not available from all of our patients. Paraffin embedded biopsy specimens from cases and BBD controls have been retrieved from the CPMC Pathology Department. Tissue sections from these specimen blocks have been cut with a microtome and stored on glass slides (10-20 slides for immunohistochemical analysis) or in plastic vials (for future DNA extraction). One slide from each patient was hematoxylin and eosin (H&E) stained to provide a histologic reference.

This archive of paired blood and tissue samples and associated questionnaire data and pathology reports is an invaluable resource that supports our current research and will form the basis of future projects that can be conducted in a timely and efficient manner.

2. Laboratory Component

The analyses of biological samples for PAH-, aromatic-, and smoking related-DNA adducts is complete, as is the immunohistochemical analysis for p53. Analysis of mononuclear white blood cells for HA-DNA adducts samples was halted in year two because none of the first 150 samples had detectable levels. The remaining funds allocated for HA-DNA adduct analysis were used to partially support the laboratory analysis of xenobiotic metabolism genes (GSTM1, NAT2 and CYP1A1). The statistical analyses of the biomarker data are presented below.

a. Postlabelling Analysis of MWBC

Mononuclear white blood cell DNA was analyzed for the presence of PAH-, smoking related-, and heterocyclic amine-DNA adducts by ^{32}P postlabelling methods in Dr. Phillips' lab. For PAH-DNA adducts results from a total of 296 subjects were returned to us from Dr. Phillips' lab. In a simple analysis of means there was no difference in adduct levels in MWBC by case-control status (see Table 3). Further analyses in subsets defined by menopausal status and current smoking status, did not alter these results.

Table 3. Mean MWBC Adduct levels by Case-Control Status

	Mean (SD)	Geometric Mean* (SD)	N
Healthy Controls	5.55 (3.13)	4.80 (1.73)	119
BBD Controls	5.53 (3.04)	4.84 (1.67)	82
Cases	5.05 (2.54)	4.48 (1.65)	95

* P=0.54

Adduct data were further analyzed in logistic regression models with subjects assigned to exposure groups based on quartiles of the distribution seen in the healthy controls. The first quartile having the lowest adduct levels and the fourth the highest adduct levels. In analyses that did not control for potential confounding factors, increasing adduct quartiles were not associated with case-control status (see Table 4).

Table 4. Univariate Analyses of Adducts by Quartile and Case-Control Status

	Cases Vs. BBD Controls (n=177) OR (95% CI)	Cases Vs. Healthy Controls (n=214) OR (95% CI)
Quartile 1	1	1
Quartile 2	0.76 (0.34-1.72)	1.09 (0.51-2.30)
Quartile 3	0.87 (0.36-2.09)	0.77 (0.36-1.67)
Quartile 4	0.71 (0.29-1.70)	0.75 (0.34-1.65)

Multivariate logistic regression models were implemented that controlled for the known breast cancer risk factors described above. The batch in which a sample was analyzed was treated as a potential confounder and controlled for in the multivariate models by entering variables for batch into the models. Adduct quartile odds ratios are presented in Table 5, and as in the univariate analyses there was no association between adduct quartiles and case-control status regardless of which control group was used. Further subgroup analyses defined by menopausal status and active smoking status did not reveal any associations between adduct levels and case-control status.

Table 5. Association Between MWBC Adduct Levels and Case Control Status, Controlling for Breast Cancer Risk Factors

Adducts	Cases Vs. BBD Controls (n=167) Odds Ratio (95% CI)	Cases Vs. Healthy Controls (n=206) Odds Ratio (95% CI)
Quartile 1	1	1
Quartile 2	0.56 (0.21-1.47)	1.08 (0.48-2.44)
Quartile 3	0.78 (0.28-2.22)	0.75 (0.32-1.74)
Quartile 4	0.89 (0.30-2.68)	0.66 (0.27-1.62)

The correlation between aromatic-DNA adducts measured in mononuclear white blood cells and PAH-DNA adduct levels measured in breast tissue was assessed. Among cases, MWBC adduct levels were not correlated with PAH-DNA adducts in tumor tissue ($r = -0.05$, $p=0.67$). Likewise no correlation was found between MWBC adducts and PAH-DNA adducts in normal breast tissue adjacent to the tumor ($r = -0.04$, $p=0.76$). Among the benign breast disease controls the correlation between tissue and blood adducts was –0.08, $p=0.45$. These results clearly suggest that adduct levels measured in white blood cells by postlabelling are not relevant to PAH-DNA adduct levels measured in breast tissue at the time of surgery. The poor correlation may reflect differing half lives of adducts in white blood cells compared to breast epithelium, or that the postlabelling assay measures a wider spectrum of adducts that includes species other than PAH.

b. The Presence of a DRZ and Smoking Related Adducts.

In the postlabelling assay, after adducts have been chromatographically separated a dark diagonal radioactive zone (DRZ) can sometimes been seen in autoradiographs of the TLC plates. Previous work has shown an association between the presence of a DRZ and smoking status, and as a result the DRZ has been thought to indicate the presence of smoking related-DNA adducts [2,5,6]. For the case-control study the presence of a DRZ was conceptualized as a qualitative marker indicating which women had been exposed to a bioeffective dose of cigarette smoke constituents.

TLC plates from 34 of the study subjects showed the presence of a DRZ, 18 of whom were healthy controls, 10 were BBD controls and 6 were cases. In univariate case controls analyses the presence of the DRZ was inversely associated with breast cancer case-control status, with borderline significance ($p=0.051$ by Fisher's exact) in the comparison between cases and healthy controls (see Table 6). Although restricted by the small number of cases positive for the DRZ the same trends were seen after stratification by menopausal status and in the sub-group exposed to tobacco smoke.

Table 6. Univariate Analyses of DRZ and Case-Control Status

	Cases Vs. BBD Controls (n=177) Odds Ratio (95% CI)	Cases Vs. Healthy Controls (n=214) Odds Ratio (95% CI)
DRZ Absent	1	1
DRZ Present	0.49 (0.17-1.40)	0.38 (0.14-1.00)

The presence of a DRZ was further analyzed in logistic regression models that controlled for sample batch and known breast cancer risk factors. In these analyses the odds ratios remained below unity and the odds ratio in the comparison of cases versus healthy controls was statistically significant. Comparing cases to BBD controls the odds ratio was 0.61 (95% CI 0.18-2.08) and in the analysis comparing cases to healthy controls the odds ratio was 0.27 (95% CI 0.09-0.76). It is important to note that this analysis involved a large number of variables and a small number of subjects who were positive for the presence of a DRZ. Therefore larger study groups are needed to resolve this question.

c. **Heterocyclic Amine-DNA Adducts in Mononuclear White Blood Cells**

Mononuclear white blood cells from 150 subjects (50 cases, 50 BBD controls and 50 healthy controls) were assayed for heterocyclic amines using the ATP deficient variation of the ^{32}P-postlabelling assay. None of the samples had detectable levels of heterocyclic amine-DNA adducts, and this assay was discontinued after year 2 (as stated in previous progress reports). Remaining funds originally allocated for this assay were used to partially support the analysis of GSTM1, NAT2 and CYP1A1 genotypes.

d. **PAH-DNA Adduct Analysis by Immunohistochemistry in Breast Tissue**

An immunohistochemistry assay was used in our laboratory to analyze the paraffin-embedded tissue samples for PAH-DNA adducts. The assay utilizes a sensitive polyclonal antiserum, developed in Dr. Regina Santella's laboratory at the Columbia School of Public Health, that is highly sensitive and specific for PAH-DNA adducts [7-9]. Stained slides are analyzed on a Becton Dickson Cell Analysis System (CAS 200) which measures the Optical Density (OD) of the staining on the slides. The OD results provide a quantitative measure of the amount of antibody staining and thus of PAH-DNA adduct levels.

All of the available paraffin embedded tissue samples from cases and BBD controls have been assayed using this method. PAH-DNA adduct data in breast tissue were available from 100 cases and from 105 controls. Additionally, 89 tissue sections of normal tissue adjacent to the tumor have been analyzed. Statistical data analyses of tissue adduct levels have been completed and a manuscript has been submitted to the Journal of the National Cancer Institute. The unadjusted arithmetic mean adduct level was significantly higher in the tumor tissue (mean=0.47) compared to the benign tissue (mean=0.38, p=0.02), and marginally higher in nontumor tissue from cases (mean=0.43) versus benign tissue (p=0.13). Reflecting the right skewed distribution of adducts, unadjusted geometric mean adduct levels were only slightly higher in tumor samples from cases compared to controls (geometric means 0.39 and 0.34 respectively, p= 0.1) and in nontumor tissue from cases versus benign tissue (geometric means 0.37 and 0.34 respectively, p= 0.2). Regression analysis showed that adduct levels in tumor and nontumor tissue from breast cancer patients were similar (slope=0.66, r=0.55, P<0.001 for 85 subjects). Adduct levels were dichotomized into high versus low using a cutoff of one standard deviation above the mean level seen in the BBD controls. Twenty- seven (27%) of the cases had high adduct levels (tumor tissue) compared to 14 (13%) of the controls (OR=2.40, 95% CI 1.18-4.92, n=205), without adjusting for potential confounders. Controlling for common PAH exposure sources (current active smoking, current ETS exposure, heavy charbroiled meat consumption) and known risk factors elevated levels of PAH-DNA adducts were significantly associated with breast cancer case-control status (OR = 2.56, 95% CI 1.05 - 6.24, n=190). Interestingly PAH-DNA adduct levels in tumor tissue were significantly positively associated with estrogen receptor status, suggesting that hormone dependence may be associated with increased adduct formation.

e. ***p53* Analysis**

Immunohistochemical techniques have been used to initially screen for *p53* mutations. While wild type p53 has a very short half-life, many mutant p53 proteins have an increased stability leading to an accumulation of protein that is detectable using immunohistochemical techniques [10,11]. Immunohistochemical detection has been found to correlate well with SSCP/PCR techniques for mutant detection [12] [13].

In the literature, samples have been variously scored positive or negative for *p53* accumulation based on the intensity of staining, the percentage of cells that stain positive for p53, or a combination of these two measures [14] [12,15] [16]. Both of these indexes can be measured on the CAS 200 system by using the Quantitative Nuclear Antigen Program (QNAP). QNAP incorporates a thresholding system that determines the percentage of nuclei that have been stained darker than a certain optical density level. This threshold is set by determining the optical density of nuclei that have been assayed using an immunohistochemical protocol that omits the primary p53 antibody. This provides an objective measure of the percentage of cell nuclei that have been stained darker than the background level of the assay.

Immunohistochemical analysis has been completed on all the tissue sections received from the Pathology Department. P53 data was available from benign tissue sections from 105 BBD controls, from tumor sections from 101 cases and from normal tissue from 87 cases. As expected, the mean number of nuclei staining positive for p53 in tumor tissue from cases was higher than in normal tissue from controls (12.12 %, SD=14.80 vs. 5.99 %, SD=5.43, respectively, p=0.02). Using a cut-off of 10% to designate extensive staining and the presumed presence of mutation, tumor tissue from cases was more likely to extensive staining than benign tissue from controls (40% vs. 21% respectively, p=0.02). The prevalence of tumor samples with extensive staining for p53 is consistent with the published literature [17].

The relationships between adduct levels and p53 expression (as the continuous variable percentage of cells staining for p53) in the three types of breast tissue were evaluated. Data on adducts and p53 were available in tumor tissue from 99 cases, in normal tissue from 81 cases, and in benign tissue from 105 controls. Adducts and p53 expression were not associated in tumor tissue or normal tissue from cases, but were associated with borderline significance in benign tissue from controls (see Table 7). Further control for exposure related variables and breast cancer risk factors did not markedly change the results, although the relationship in benign tissue became a little stronger after control for known breast cancer risk factors (slope = 1.64, p=0.06).

Table 7. Univariate Linear Regression Analyses of Tissue PAH-DNA Adduct Levels and p53 Expression.

	Slope, N	P value
Tumor tissue from cases	0.36, n=99	0.49
Normal tissue from cases	-0.49, n=81	0.35
Benign tissue from controls	1.32, n=105	0.09

The relationship between tissue adducts and p53 expression in cases was also assessed by comparing the prevalence of extensive p53 staining in women with high adduct levels in tumor tissue to the prevalence among women with low adduct levels. Similar to the linear regression analysis there was no association between adducts levels in tumor tissue and the presence of extensive staining for p53 (OR = 1.0, 95% CI 0.40-2.47).

The relationship between aromatic-DNA adducts measured in mononuclear white blood cells and p53 status was also assessed. Polytomous logistic regression analyses were used to determine whether elevated adducts levels were more strongly associated with p53 positive tumors than p53 negative tumors. This approach simultaneously compares p53 positive cases to controls and p53 negative cases to controls and calculates separate odds ratios for both comparisons. The ratio of these odds ratios indicates the degree to which the exposure is more strongly associated with p53+ cases, and is a measure of etiologic heterogeneity [17]. These analyses showed that in comparison to either control group, increasing adduct quartiles were not associated with p53 positive or negative cases compared to controls (see Tables 8 and 9).

Table 8. Polytomous Logistic Regression Comparing Cases to BBD Controls, Controlling for Known Breast Cancer Risk Factors

	P53+ Cases vs. BBD Controls	P53- Cases vs. BBD Controls	
Adduct Quartiles	OR (95% CI)	OR (95% CI)	Ratio (95% CI)
1	1	1	1
2	0.35 (0.09-1.33)	0.39 (0.11-1.31)	0.92 (0.11-7.61)
3	0.77 (0.21-2.83)	0.61 (0.17-2.18)	1.26 (0.14-11.06)
4	0.88 (0.22-3.50)	0.62 (0.15-2.53)	1.42 (0.14-14.48)

Table 9. Polytomous Logistic Regression Comparing Cases to Healthy Controls, Controlling for Known Breast Cancer Risk Factors

	P53+ Cases vs. Healthy Controls	P53- Cases vs. Healthy Controls	
Adduct Quartiles	OR (95% CI)	OR (95% CI)	Ratio (95% CI)
1	1	1	1
2	0.56 (0.17-1.86)	1.17 (0.44-1.31)	0.48 (0.08-2.79)
3	0.69 (0.22-2.20)	0.85 (0.31-2.33)	0.81 (0.14-4.61)
4	0.45 (0.13-1.59)	0.58 (0.18-1.83)	0.77 (0.11-5.28)

f. **Additional Studies of Genetic Susceptibility and Oncoproteins**

Over the course of the study we have used funds available from other sources and from the reallocation of funds originally earmarked for HA-DNA adducts to analyze stored tissue and blood samples for other markers such as: genetic susceptibility, exposure to organochlorine compounds and various oncoproteins. These markers were chosen either because they would complement the markers used in our main study or for use as pilot data to support additional grant proposals.

i. **Complementary Markers: Polymorphisms in Metabolic Genes**

Over the course of the study we have analyzed white blood cell DNA from our breast cancer cases and controls for polymorphisms in NAT2, GSTM1 and CYP1A1, genes that mediate the metabolism/detoxification of the environmental carcinogens under study [18-20 21-24].

We have genotyped subjects for the GTSM1 genotype using DNA from the collected white blood cells. Of the 365 samples assayed, 333 could be successfully amplified by PCR. We have completed the statistical analysis of genotype, PAH-DNA adduct levels in tissue and case-control status in cases and BBD controls. Data on *GSTM1* genotype were available from 95 cases and 87 BBD controls and data on both *GSTM1* status and PAH-DNA adduct levels were available from 84 controls and 83 cases. GSTM1 genotype was predictive of adduct levels in tumor tissue and normal tissue from cases but not in tissue from BBD controls (see Table 10). There were no case-control differences in tissue adduct levels among women with the GSTM1 +/+, +/- genotypes, but among women with the GSTM1 -/- genotype adduct levels were significantly higher in tumor tissue from cases compared to levels in benign tissue from controls (see Table 10).

Table 10. PAH-DNA Adducts in Breast Tissue by *GSTM1* Genotype

	GSTM1 +/+, +/-		*GTSM1* -/-		
Cases	Mean[1] (SD)	Geometric Mean (SD)	Mean (SD)	Geometric Mean (SD)	
Tumor	0.39 (0.21) n=43	0.33 (1.74)	0.61 (0.37) n=40	0.50 (1.93)	P=0.003[2]
Nontumor	0.37 (0.18) n=39	0.34 (1.61)	0.50 (0.30) n=37	0.43 (1.82)	P=0.059
Benign controls	0.37 (0.18) n=41	0.34 (1.57)	0.41 (0.18) n=43	0.37 (1.59)	P=0.341

1 In optical density units

2 P values based on ln transformed data (geometric means), comparing adduct levels in subjects who are *GSTM1* +/+, +/- vs. those who are *GSTM1*-/-.

It appeared that there was an interaction between genotype, adduct levels and case-control status. A linear regression model was implemented with PAH-DNA adduct levels in tumor tissue from cases and in benign tissue from controls as the dependent variable, and case-control status, GSTM1 genotype, and a term for the interaction of these two effects as the independent variables. In this model the interaction term for the joint effects of genotype and case-control status was of borderline significance (β = 0.31, p=0.06). In a multiple linear regression model that also controlled for known breast cancer risk factors and PAH exposures, the interaction term for the joint effects of *GSTM1* and case-control status was significant (β = 0.25, p=0.002).

GSTM1 genotype did not predict aromatic-DNA adduct levels in mononuclear white blood cells. In univariate linear regression models with adducts as the outcome, the regression coefficients for GSTM1 did not significantly differ from zero (Cases β=-0.1 p=0.36, BBD Controls β= 0.002, p=0.99, Healthy Controls β=0.42, p=0.18). In multiple linear regression models controlling for batch in which the sample was assayed, season in which the blood samples was drawn, age, ethnicity, current smoking status, current ETS exposure and high charred meat consumption, GSTM1 remained unassociated with adduct levels in each of the study groups.

In univariate analysis *GSTM1* genotype as a sole marker of metabolic susceptibility was not associated with case-control status (Cases vs. Benign OR = 0.92, 95% CI 0.51 – 1.64, Cases vs. Healthy OR = 1.21, 95% CI 0.51-1.64). After controlling for age, ethnicity, breast feeding status, parity, age at menarche, age at first birth, family history of breast cancer and current alcohol consumption, *GSTM1* genotype remained unassociated with breast cancer case-control status (Cases vs. BBD Controls OR = 0.73, 95% CI 0.37 – 1.44, Cases vs. Healthy Controls OR = 1.21, 95% CI 0.70-2.10). The associations between

GSTM1 genotype status and prognostic factors recorded in the pathology reports were also assessed. *GSTM1* genotype was not associated with tumor size, stage or erbB-2 expression. However, the *GSTM1* null genotype was associated with increased estrogen receptor expression. A manuscript based on these results has been submitted to the journal Cancer Epidemiology Biomarkers and Prevention.

Our laboratory has just completed the analyses of the NAT2 and CYP1A1 genotypes using WBC DNA. The association between individual genotypes and breast cancer case-control status and the formation of adducts in blood and tissue specimens will be analyzed, as will be the effect of combinations of genotypes.

ii. Pilot Studies: Development and Validation of Tumor Derived Bloodborne Markers (cyclin D1 and ras p21) Predictive of Breast Cancer Status

The *Cyclin D1* gene is thought to be a proto-oncogene, which, when aberrantly expressed, leads to loss of normal growth control [25 26]. *Cyclin D1* overexpression is commonly seen in squamous cell carcinomas of the head, neck, lung and esophagus, as well as in breast carcinomas [25-28]. Using immunohistochemical techniques, *cyclin D1* protein overexpression can be detected in 50-60% of paraffin embedded breast tumor specimens, and overexpression is associated with estrogen receptor positive tumors and a better prognosis [26,28,28,29,29].

In years two and three, 67 tissue sections from 25 BBD controls and 23 cases were assayed for cyclin D1 expression. Blood plasma samples were available from 24 of the BBD controls and 19 of the cases; and in year four these were analyzed for the presence of cyclin D1 using a newly developed Western blot assay. Additionally, blood plasma samples were analyzed from 15 healthy controls who were individually matched on age and ethnicity to the first 15 cases. Cyclin D1 was detected in 21 of the blood samples, with the following distribution among cases and controls: 3 of 15 healthy controls (20%), 6 of 24 of BBD controls (25%), and 12 of 19 cases (63%), p=0.011 by Chi-square. The odds ratio for *cyclin D1* and breast cancer (cases versus BBD controls) was 5.14, 95% C.I. 1.38-19.12 (p=0.012) and for cases versus healthy controls it was 6.86, 95% CI 1.43-33.01 (p=0.012). There was no correlation between the presence of cyclin D1 in blood plasma and the level of cyclin D1 expression in tumor tissue; however the small number of subjects limited this analysis.

Since the Western blots were scored for the presence of absence of cyclin D1 bands with the unaided eye, it is not possible to determine objectively whether the bands seen in the controls were lighter or smaller than the bands seen in cases. We are currently developing an ELISA assay for cyclin D1 that will give us a quantitative measure of the protein concentration in the blood samples. We expect that such a quantitative measure will allow us to set a cutoff concentration that will better discriminate between cases and controls and reduce the false positive rate. This strategy has successfully been used to assay for blood levels of the erbB-2 extracellular domain (see for example reference 30).

We have recently been awarded an R0-1 grant (Dr. D. Tang P.I.) to analyze all of the stored blood and tissue samples for cyclin D1. The project will further develop the Western blot method and will allow us to complete the development of the ELISA assay. The project will assess whether the presence of cyclin D1 in blood samples is associated with breast cancer case-control status in the full study population. It will also assess whether cyclin D1 overexpression in tumor tissue is associated with the presence of cyclin D1 in blood samples. Finally the project will assess whether the presence of cyclin D1 in blood samples is associated with tumor characteristics such estrogen receptor status.

In a related pilot project conducted in year two we used an existing technique for detecting *ras* p21 protein in blood samples to assay stored samples. It has been shown in other cancers that *ras* p21 can be detected in blood samples from patients who have tumors that have mutated or overexpressed *ras* [31,32]. Since breast tumors commonly overexpress *ras* p21, we investigated whether breast cancer patients exhibited increased blood levels of *ras* and whether these increased levels predicted case-control status. The development of bloodborne markers that can predict breast cancer status may allow for new strategies to detect breast tumors and could be useful in post-surgical follow-up of patients [33-35].

Blood samples from the first 94 subjects enrolled in the case-control study were analyzed for ras p21. None of the women had received treatment for either breast cancer or benign breast disease at the time the blood sample was drawn. They included 34 cases (5 patients with diagnoses of DCIS, 8 with invasive lobular cancer and 21 with invasive ductal cancer), 26 benign breast disease (BBD) controls and 34 healthy controls. Wild type (non-mutated) ras p21 protein was detected in 34 of 94 (36%) plasma samples although many of the bands were quite faint. Mutant ras was not detected in any of the samples. Cases had significantly higher levels of ras p21 in their blood samples than either of the control groups (see Table 11). When the lanes were classified as having normal or elevated levels of ras p21, samples from 17 of the 94 (18%) individuals were positive for elevated levels of ras p21. The prevalence of plasma samples with elevated levels was higher in cases compared to controls (see Table 11). Because the two control groups had similar mean, median and prevalence values, they were combined. In this analysis, the presence of elevated levels of ras p21 was found to significantly predict case-control status (OR=3.2, 95% CI 1.1-9.3, p=0.03). Although analyses were restricted by the small sample size of this pilot study, ethnicity appeared to modify the relationship between case-control status and the presence of ras p21 in blood samples. Comparing cases to the combined control group, ras p21 was strongly associated with case-control status among Caucasian women (OR = 11.0, 95% CI 1.3-95.3, p=0.01), but not among non-Caucasian women (OR = 0.86, 95% CI 0.01-8.5, p= 0.9).

Table 11. Mean and Median ras p21 Level and Prevalence of Elevated ras p21 Levels by Study Group.

Study Group	Mean (IPU)	Median* (IPU)	Subjects with Elevated Ras p21 Levels**	N
Breast Cancer Cases	13.63	7.04	10 (29%)	34
BBD Controls	5.65	0.00	3 (12%)	26
Healthy Controls	5.24	0.00	4 (12%)	34

* P= 0.03 for differences across study groups by Kruskal-Wallis 1-Way ANOVA

** P=0.12 the cases versus either of the control groups by Fisher's Exact and p=0.03 for cases versus the combined control groups by Chi Square Test.

A manuscript based on these results has been accepted for publication by the journal Cancer Epidemiology Biomarkers & Prevention. In spring of 2000 we plan to submit an R0-1 grant to the NCI to assay for ras p21 in blood and tissue samples from all of the enrolled subjects. This project will be a major step towards developing a blood test that is predictive for breast cancer.

iii. Pilot Study: Blood DDE Levels Among Latina Women in Northern Manhattan

A portion of our present breast cancer study sample is drawn from a large Latina population that immigrated to the United States from the Dominican Republic and settled in Northern Manhattan. Many of these subjects recall intensive government programs of DDT application that included the spraying of homes and aerial spraying of agricultural areas. Since prior studies have found an association between blood levels of DDE (a metabolite of DDT) and breast cancer it was of interest to determine whether these intensive exposures have led to increased blood DDE levels in this portion of our study population. In year three, under separate funding, blood plasma samples from 19 healthy controls, 15 benign breast disease controls and 14 cases were analyzed for DDE levels using gas chromatography with electron detection. DDE levels in these Dominican subjects ranged from 0.2 - 113.2 ppm with a mean of 18.3 ppm. No significant differences could be detected between cases and controls, possibly due to the small number of subjects.

DDE levels in these subjects were compared to levels previously measured in 24 healthy non-Dominican women from the New York Metropolitan region who were treated at the Columbia-Presbyterian Medical Center for breast cysts. DDE levels were 3.22 times higher in the Dominican women than in the non-Dominican women (14.76 ppm vs. 4.56 ppm, p=0.005). Additionally, DDE levels were significantly associated with the number of years the women had lived in the Dominican Republic, with age and with the number of months the women breast fed their children. The increased body burden of DDE seen in Dominican women suggests that they would be a good model population in which to study the possible links between DDE and health outcomes, including breast cancer [3].

The blood samples were also analyzed for DDT, PCB (total and 21 congeners), hexochloro-benzene, and Mirex (1,200 determinations in total). Statistical analyses of these compounds has not yet been completed.

KEY RESEARCH ACCOMPLISHMENTS

- Provided evidence supporting the hypothesis that breast cancer has, in part, an environmental etiology and is thus preventable.

    - Generated data indicating that the formation of PAH- related DNA damage in breast tissue was associated with breast cancer case-control status, suggesting that these environmental carcinogens contribute to breast cancer.

    - Generated data indicating that PAH-DNA adduct levels in tumor tissue are associated with estrogen receptor expression.

    - Generated data indicating that in women with breast cancer the inability to metabolize PAH through the GSTM1 pathway is associated with increased PAH-DNA adduct levels, suggesting that gene-environment interactions may play a role in breast cancer development.

- Generated data suggesting that the role of PAH in breast cancer etiology may not be related to p53 mutation.

- Demonstrated that measurements of adduct levels in peripheral white blood cells may not be a good surrogate for similar measurements in breast tumor tissue. This finding provides important guidance for other investigators and the design of future studies.

- Showed that DNA damage from heterocyclic amines does not reach detectable levels in women with dietary practices within the normal range seen in the North Eastern United States.

- Developed pilot data showing that oncoproteins (ras p21 and cyclin D1) overexpressed by breast tumors are detectable in blood samples from cases, and that the presence of these proteins in blood samples is predictive of cases-control status. This work suggests that a blood test based on these and similar markers can be developed that will provide patients and physicians with new options for early detection and management of breast cancer.

- Demonstrated that past intensive exposures to DDT among immigrants from the Dominican Republic resulted in increased body burdens of DDE that are still detectable today and are three fold higher than those seen in non-immigrant women from the New York Metropolitan Area. This suggests that Dominican women maybe a good model population in which to study the effects of DDE.

- Established a repository of breast tissue and blood samples linked to pathology data, and a database with detailed questionnaire data on lifestyle and known breast cancer risk factors.

REPORTABLE OUTCOMES

1. Manuscripts

Rundle, A., Tang, D., /Zhou, J., Cho, S., Perera, F. The effect of glutathione S-transferase-M1 genotype on polycyclic aromatic hydrocarbon (PAH)-DNA adducts in breast tissue on breast cancer risk. Submitted to Cancer Epidemiology Biomarkers & Prevention

Rundle, A., Tang, D., Hibshoosh, H., Estabrook, A., Schnabel, F., Cao, WF., Grumet, S., Della Rocca, A., Perera, F. Genetic damage in breast tissue from polycyclic aromatic hydrocarbons is associated with breast cancer. Submitted to the Journal of the National Cancer Institute

Rundle, A., Tang, D., Brandt-Rauf, P., Zhou, J., Perera, J. Association between the ras p21 oncoprotein in blood samples and breast cancer. Accepted for publication at Cancer Epidemiology Biomarkers & Prevention

Tang, D., Rundle, A, Chen, Q.W., Zhou, J., and Brandt-Rauf, P. Association between *cyclin D1* measured in blood plasma samples and breast cancer case-control status. Submitted to Environmental Mutagenesis

Blackwood, A., Wolff, M., Rundle, A., Estrabook, A., Schnabel, F., Mooney, L. A., Rivera, M., Channing, K. M., Perera, F. P. (1998) Organochlorine compounds (DDE and PCB) in plasma and breast cyst fluid of women with benign breast disease. Cancer Epidemiol Biomarkers Prev 7:579-583.

Perera, F. P. (1997) Environment and cancer: who are susceptible? Science 278:1068-73.

Perera, F. P. (1996) Molecular epidemiology: insights into cancer susceptibility, risk assessment, and prevention. JNCI 88:496-508.

Perera, F., Mooney, L., Dickey, C., Santella, R., Bell, D., Blaner, W., Tang, D., Whyatt, R. (1996) Molecular epidemiology in environmental carcinogenesis. Environ Health Perspect 104:441-443.

Perera, F. P., Blaner, W., Mooney, LV. A., (1996) Molecular epidemiologic methods in cancer chemoprevention. European J. Cancer Prevention 5 Suppl 2:19-25.

Perera, F. P. (1995) Molecular epidemiology and prevention of cancer. Environ Health Perspect 103 Suppl 8:233-235.

2. Abstracts

Rundle, A., Tang, D., Zhou, J.Z., Perera, FP. PAH-DNA adduct levels in breast tissue from women with breast cancer and benign breast disease. Submitted for the 2000 AACR Meeting.

Tang, D., Rundle, A., Zhou, J.Z., Chen, Q.W., Brandt-Rauf, P., Perera, F. Association between cyclin D1 measured in blood plasma samples and breast cancer case-control status. Proceedings of the 1999 AACR Meeting.

Rundle, A., Tang, D.L., Zhou, J., Brandt-Rauf, P., Perera, F. Detection of *RAS* p21in Blood Samples from Women with Breast Cancer. Proceedings of The Department of Defense Breast Cancer Research Program Meeting, Era of Hope, 1997.

Shao, Q., Tang, D., Rundle, A., Zhou, J. and Perera, P. Association between p53, cyclin D1 and erbB-2 protein and breast cancer. Proceedings of the 1997 ASPO Annual Meeting.

Rundle, A., Tang, D.L., Zhou, J., Brandt-Rauf, P., Perera, F. Detection of *Ras* p21 Oncoprotein in Plasma Samples from Women with Breast Cancer and Controls. Proceedings of the 1996 APHA Annual Meeting.

Perera, F.P., Estabrook, A., Hewer, A., Channing, K., Rundle, A., Mooney, L., Whyatt, R., Phillips, D.H. Aromatic Carcinogen DNA Adducts in Human Breast Tissue. Proceedings of 1994 AACR Meeting.

3. Presentations

Perera, FP Environment-Gene Interactions Elaborated by Molecular Epidemiology. Gordon Research Conference on Genetic Toxicology 1999

Rundle, A., Biological Markers of Environmental Carcinogens in Breast Cancer, New Investigators Workshop, American Society for Preventive Oncology Annual Meeting 1998.

Rundle A., Detection of Ras p21 Oncoprotein in Plasma Samples from Women with Breast Cancer and Controls, APHA Annual Meeting, 11/20/96.

Rundle, A., Research on Environmental Risk Factors for Breast Cancer. WAR: Women at Risk Lecture Series, Columbia-Presbyterian Medical Center, 10/23/95.

Rundle A., Detection of Ras p21 Oncoprotein in Plasma Samples from Women with Breast Cancer and Controls, APHA Annual Meeting, 11/20/96.

4. Patents

The results on the use of blood levels of cyclin D1 as a marker of breast cancer have been submitted to the Columbia Innovation Enterprise Office for patent evaluation.

5. Degrees

Andrew Rundle, MPH and Dr.P.H., Columbia School of Public Health
Claribel Blake, MPH, Columbia School of Public Health
Surah Grumet, MPH, Columbia School of Public Health
Amy Della Rocca, MPH and Masters of Nursing

6. Tissue Repository

A repository of tissue samples and aliquoted blood samples has been established. Blood samples from cases, BBD controls, and healthy controls were separated into plasma, red blood cells, and white blood cells and stored at -80°C. Paraffin embedded tissue blocks were retrieved from the Pathology department and 5 micron thick slices were cut and mounted on immunoblank glasses slides suitable for immunohistochemical analyses. Tissue sections (between 2 and 12 per subject) are available from breast tumor tissue from cases, normal breast tissue from cases, and benign tissue from BBD controls. Tissue and blood samples are linked through ID numbers to pathology reports, and in depth questionnaire data on lifestyle, environmental exposures and known breast cancer risk factors.

7. Funding Applied For

Based on our pilot work showing that the presence of cyclin D1 in blood samples is associated with breast cancer case-control status we applied for and received funding for an R0-1 project (Dr. Tang PI). The project will assay cyclin D1 in stored blood samples from all of the enrolled subjects and in tumor tissue sections from cases. The goal of the study is to determine whether cyclin D1 in blood samples is predictive of breast cancer status in the full group of subjects, and to determine whether it is more predictive of estrogen receptor positive breast cancer. A second goal is to determine whether cyclin D1 expression in tumor tissue is associated with the presence of cyclin D1 in blood, and whether this association is modified by such factors as tumor size or stage.

We are currently developing collaborative projects using the stored tissue and blood samples that will be included in a breast cancer SPORE proposal being submitted by the Columbia Comprehensive Cancer Center.

In the spring of 2000 we plan to submit an R0-1 proposal based on our pilot work on the measurement of ras p21 in blood. As described above in our pilot study the presence of this oncoprotein marker in blood was strongly associated with breast cancer case-control status, and may prove useful as the basis for a breast cancer predictive blood test. In a manner similar to our recently funded work on cyclin D1 we plan to further validate this marker by assaying for it in the stored blood samples from cases and controls and in tumor tissue from cases.

In the spring of 2000 we also plan to submit an RO-1 proposal to reopen the study to patient recruitment. This will increase our statistical power to investigate gene-environment interactions as they relate to adduct levels in breast tissue.

8. **Employment or research opportunities received on training supported by this award.**
Andrew Rundle earned a Doctorate in Public Health while working on this project and subsequently applied for and received a junior faculty position in the Division of Epidemiology at the Joseph L. Mailman School of Public Health. He will continue working with Dr. Perera studying the etiology of breast cancer and developing and validating new approaches for the early detection and management of breast cancer. He will also be conducting epidemiologic investigations into the etiology of cancer with faculty in the Division of Epidemiology.

CONCLUSION

Breast cancer will strike some 178,000 women this year in the United States, exacting a terrible personal and public health toll. Although a number of risk factors have been elucidated, they only explain about 50% of breast cancer and have yielded few primary prevention programs. A number of investigators have begun to look at environmental factors as possible causes of this disease. This project has been extremely fruitful and has yielded important data supporting the hypothesis that the widespread environmental contaminants, PAH, play a role in breast cancer etiology. Further, our investigations into genetic polymorphisms in GSTM1 suggest that this gene plays a role in protecting breast tissue from PAH-induced DNA damage, and that gene-environment interactions may put women at risk for developing breast cancer. Since our environment is modifiable, these finding, if replicated, suggest that prevention programs can be implemented that will reduce the incidence of this disease.

The study also provided the infrastructure for supporting many studies to come. The tissue bank linked to the pathology and questionnaire data provides an invaluable resource that will allow us and other investigators to a multitude of hypotheses regarding breast cancer etiology and to develop new markers that will be of use in managing breast cancer. This tissue bank has already yielded dividends. Pilot data showing that the presence of cyclin D1 in blood associated with breast cancer status has been used to secure a new RO-1 project that will further develop and validate this marker. Several other RO-1 proposals are planned for the spring of 2000 and the tissue bank will be part of an upcoming breast cancer SPORE proposal. This repository will be a continuing resource that will greatly further our understanding of the etiology of breast cancer, pointing the way towards new means of prevention.

REFERENCES

1. ACS. Cancer Facts and Figures - 1999. *Atlanta: American Cancer Society.* 1999.

2. Perera FP, Estabrook A, Hewer A, et al. Carcinogen-DNA adducts in human breast tissue. *Cancer Epidemiol Biomarker Prev.* 1995;4:233-238.

3. Wolff MS, Toniolo PG, Lee EW, Rivera M, Dubin N. Blood levels of organochlorine residues and risk of breast cancer. *J Natl Cancer Inst.* 1993;85:648-652.

4. El-Bayoumy K. Environmental carcinogens that may be involved in human breast cancer etiology. *Chem Res Toxicol.* 1992;5:585-590.

5. Phillips DH, Hewer A, Martin CN, Garner RG, King MM. Correlation of DNA adduct levels in human lung with cigarette smoking. *Nature.* 1988;336:790-792.

6. Li D, Wang M, Dhingra K, Hittelman WN. Aromatic DNA addducts in adjacent tissues of breast cancer patients: clues to breast cancer etiology. *Cancer Res.* 1996;56:287-93.

7. Hsu T, Zhang Y, and Santella RM. Immunoperoxidase Quantitation of 4-Aminobipheny- and Polycylic Aromatic Hydrocarbon-DNA Adducts in Exfoliated Oral and Urothelial Cells of Smokers and Nonsmokers. *Cancer Epidemiology, Biomakers & Prevention*. 1997;6:193-199.

8. Zhang Y, Weksler B, Wang L, Schwartz J, Santella R. Immunohistochemical detection of polycyclic aromatic hydrocarbon-DNA damage in human blood vessels of smokers and non-smokers. *Atherosclerosis.* 1998;140(2):325-31.

9. Romano G, Sgambato A, Boninsegna A, et al. Evaluation of Polycyclic Aromatic Hydrocarbon-DNA Adducts in Exfoliated Oral Cells by an Immunohistochemical Assay. *Cancer Epidemiol Biomarkers Prev.* 1999;8(1):91-96.

10. Coles C, Condie A, Chetty U, Steel CM, Evans HJ, Prosser J. p53 mutations in breast cancer. *Cancer Res.* 1992;52:5291-5298.

11. Davidoff AM, Humphrey PA, Iglehart JD, Marks JR. Genetic basis for p53 overexpression in human breast cancer. *Proc Natl Acad Sci USA.* 1991;88:5006-5010.

12. Cordon-Cardo C, Dalbagni G, Guillermo S, et al. p53 mutations in human bladder cancer: Genotypic versus phenotypic patterns. *Int. J. Cancer.* 1994;56:347-353.

13. Umekita Y, Kobayashi K, Saheki T, Yoshida H. Nuclear accumulation of p53 protein correlates with mutations in the p53 gene on archival paraffin-embedded tissues of human breast cancer. *Japan J Cancer Res.* 1994;85:825-30.

14. Allred DG, Clark GM, Elledge R, et al. Association of p53 protein expression with tumor cell

proliferation rate and clinical outcome in node-negative breast cancer. *JNCI.* 1993;85:200-206.

15. Koutselini H, Malliri A, Field JK, Spandidos DA. p53 expression in cytologic specimens from benign and malignant breast lesions. *Anticancer Res.* 1991;11:1415-1420.

16. Ostrowski JL, Sawan A, Henry L, et al. p53 expression in human breast cancer related to survival and prognostic factors: An immunohistochemical study. *J Pathol.* 1991;164:75-81.

17. Gammon M, Hibshoosh H, Terry M, et al. Cigarette smoking and other risk factors in relation to p53 expression in breast cancer among young women. *Cancer Epidemiol Biomarker Prev.* 1999;8(3):255-263.

18. Blum M, Demierre A, Grant DM, Heim M, Meyer UA. Molecular mechanism of slow acetylation of drugs and carcinogens in humans. *Med Sciences.* 1991;88:5237-5241.

19. Kadlubar FF, Butler MA, Kaderlik KR, Chou H-C, Lang NP. Polymorphisms for aromatic amine metabolism in humans: Relevance ro human carcinogenesis. *Environ Health Perspect.* 1992;98:69-74.

20. Martell KJ, Levy GN, Weber WW. Cloned mouse N-acetyltransferases: Enzymatic properties of expressed Nat-1 and Nat-2 gene products. *Mol Pharmacol.* 1992;42:265-272.

21. Robertson IGC, Guthenberg C, Mannervik B, Jernstrom B. Differences in stereoselectivity and catalytic efficiency of three human glutathione transferases in the conjugation of glutathione with 7B, 8 a-dihydroxy-9a, 10 a-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene. *Cancer Res.* 1986;46:2220-2224.

22. Liu YH, Taylor J, Linko P, Nagorney D, Lucier G, Thompson C. Glutathione-S-transferase μ in human lymphocyte and liver: Role in modulating formation of carcinogen derived DNA adduct. *Carcinogenesis.* 1991;12:2269-2275.

23. Pelkonen O, Nebert DW. Metabolism of polycyclic aromatic hydrocarbons: etiologic role in carcinogenesis. *Pharmacological Reviews.* 1982;34:189-222.

24. Shields PG. Inherited factors and environmental exposures in cancer risk. *J Occup Med.* 1993;35:34-41.

25. Buckley M, Sweeney K, Hamilton J, et al. Expression and amplification of cyclin genes in human breast cancer. *Oncogene.* 1993;8:2127-2133.

26. Zhang S, Caamano J, Copper F, Guo X, Klein-Szanto AJP. Immunohistochemistry of Cyclin D1 in Human Breast Cancer. *Am J Clin Pathol.* 1994;102:695-698.

27. Musgrove E, Lee C, Buckley M, Sutherland R. Cyclin D1 induction in breast cancer cells shortens G1 and is sufficient for cells arrested in G1 to complete the cell cycle. *Proc. Natl. Acad. Sci.*

1994;91:8022-8026.

28. Gillet C, Fantl V, Smith R, et al. Amplification and Overexpression of Cyclin D1 in Breast Cancer Detected by Immunohistochemical Staining. *Cancer Research.* 1994;54:1812-1817.

29. Bartkova J, Lukas J, Muller H, Lutzzhoft D, Strauss M, Bartek J. Cyclin D1 Protein Expression and Function in Human Breast Cancer. *Int J Cancer.* 1994;57:353-361.

30. Andersen T, Paus E, Nesland J, McKenzie S, Borresen A. Detection of C-erbB-2 related protein in sera from breast cancer patients. Relationship to ERBB-2 gene amplification and c-erbB-2 protein overexpression in tumor. *Acta. Oncologica.* 1995;34:499-504.

31. Brandt-Rauf P.W. Oncogene proteins as biomarkers in the molecular epidemiology of occupational carcinogenesis . *Int Arch Occup Environ Health.* 1991;63:1-8.

32. DeVivo I, Marion MJ, Smith S, Carney W, Brandt-Rauf P. Mutant c-Ki-ras p21 protein in chemical carcinogenesis in humans exposed to vinyl chloride. *Cancer Causes and Control.* 1994;5:273-278.

33. Breuer B, DeVivio I, Luo JC, et al. erbB-2 and myc oncoproteins in sera and tumors of breast cancer patients. *Cancer Epidemiology, Biomarkers & Prevention.* 1994;3:63-66.

34. Isola J, Holli K, Oksa H, Teramoto Y, Kallioniemi O. Elevtaed erbB-2 Oncoprotein Levels in Preoperative and Follow-up Serum Samples Define an Aggressive Disease Course in Patients with Breast Cancer. *Cancer.* 1994;73:652-8.

35. McIntosh G, Anderson J, Milton I, et al. Determination of the prognostic value of cyclin D1 overexpression in breast cancer. *Oncogene.* 1995;11:885-891.

BIBLIOGRAPHY OF PUBLISHED PAPERS AND ABSTRACTS

Papers

Rundle, A., Tang, D., Zhou, J., Cho, S., Perera, F. The effect of glutathione S-transferase-M1 genotype on polycyclic aromatic hydrocarbon (PAH)-DNA adducts in breast tissue on breast cancer risk. Submitted to Cancer Epidemiology Biomarkers & Prevention

Rundle, A., Tang, D., Hibshoosh, H., Estabrook, A., Schnabel, F., Cao, WF., Grumet, S., Della Rocca, A., Perera, F. Genetic damage in breast tissue from polycyclic aromatic hydrocarbons is associated with breast cancer. Submitted to the Journal of the National Cancer Institute

Rundle, A., Tang, D., Brandt-Rauf, P., Zhou, J., Perera, J. Association between the ras p21 oncoprotein in blood samples and breast cancer. Accepted for publication at Cancer Epidemiology Biomarkers & Prevention

Tang, D., Rundle, A, Chen, Q.W., Zhou, J., and Brandt-Rauf, P. Association between *cyclin D1* measured in blood plasma samples and breast cancer case-control status. Submitted to Environmental Mutagenesis

Blackwood, A., Wolff, M., Rundle, A., Estrabook, A., Schnabel, F., Mooney, L. A., Rivera, M., Channing, K. M., Perera, F. P. (1998) Organochlorine compounds (DDE and PCB) in plasma and breast cyst fluid of women with benign breast disease. Cancer Epidemiol Biomarkers Prev 7:579-583.

Perera, F. P. (1997) Environment and cancer: who are susceptible? Science 278:1068-73.

Perera, F. P. (1996) Molecular epidemiology: insights into cancer susceptibility, risk assessment, and prevention. JNCI 88:496-508.

Perera, F., Mooney, L., Dickey, C., Santella, R., Bell, D., Blaner, W., Tang, D., Whyatt, R. (1996) Molecular epidemiology in environmental carcinogenesis. Environ Health Perspect 104:441-443.

Perera, F. P., Blaner, W., Mooney, LV. A., (1996) Molecular epidemiologic methods in cancer chemoprevention. European J. Cancer Prevention 5 Suppl 2:19-25.

Perera, F. P. (1995) Molecular epidemiology and prevention of cancer. Environ Health Perspect 103 Suppl 8:233-235.

Abstracts

Rundle, A., Tang, D., Zhou, J.Z., Perera, FP. PAH-DNA adduct levels in breast tissue from women with breast cancer and benign breast disease. Submitted for the 2000 AACR Meeting.

Tang, D., Rundle, A., Zhou, J.Z., Chen, Q.W., Brandt-Rauf, P., Perera, F. Association between cyclin D1 measured in blood plasma samples and breast cancer case-control status. Proceedings of the 1999 AACR Meeting.

Rundle, A., Tang, D.L., Zhou, J., Brandt-Rauf, P., Perera, F. Detection of *RAS* p21in Blood Samples from Women with Breast Cancer. Proceedings of The Department of Defense Breast Cancer Research Program Meeting, Era of Hope, 1997.

Shao, Q., Tang, D., Rundle, A., Zhou, J. and Perera, P. Association between p53, cyclin D1 and erbB-2 protein and breast cancer. Proceedings of the 1997 ASPO Annual Meeting.

Rundle, A., Tang, D.L., Zhou, J., Brandt-Rauf, P., Perera, F. Detection of *Ras* p21 Oncoprotein in Plasma Samples from Women with Breast Cancer and Controls. Proceedings of the 1996 APHA Annual Meeting.

Perera, F.P., Estabrook, A., Hewer, A., Channing, K., Rundle, A., Mooney, L., Whyatt, R., Phillips, D.H. Aromatic Carcinogen DNA Adducts in Human Breast Tissue. Proceedings of 1994 AACR Meeting.

PERSONNEL

Beato, F
Blake, C
Chen, Q
Cho, S
Della Rocca, A
Dickey, C
Fitzpatrick, E
Gaynor, M
Grullon, R
Hsu, Y
Lyn, S
Maggard, K
Mooney, L
Munoz, J
Paine, C
Perera, F
Rosado, A
Rundle, A
Shao, Q
Simon-Cerejido, G
Sohn, S
Tang, D
Taveras, L
Vanbenneku, A
Whyatt, R
Zhai, E
Zhou, J

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 21 JUN 2001

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports. Request the limited distribution statement for reports on the enclosed list be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

Reports to be changed to "Approved for public release; distribution unlimited"

Grant Number	Accession Document Number
DAMD17-94-J-4147	ADB221256
DAMD17-93-C-3098	ADB231640
DAMD17-94-J-4203	ADB221482
DAMD17-94-J-4245	ADB219584
DAMD17-94-J-4245	ADB233368
DAMD17-94-J-4191	ADB259074
DAMD17-94-J-4191	ADB248915
DAMD17-94-J-4191	ADB235877
DAMD17-94-J-4191	ADB222463
DAMD17-94-J-4271	ADB219183
DAMD17-94-J-4271	ADB233330
DAMD17-94-J-4271	ADB246547
DAMD17-94-J-4271	ADB258564
DAMD17-94-J-4251	ADB225344
DAMD17-94-J-4251	ADB234439
DAMD17-94-J-4251	ADB248851
DAMD17-94-J-4251	ADB259028
DAMD17-94-J-4499	ADB221883
DAMD17-94-J-4499	ADB233109
DAMD17-94-J-4499	ADB247447
DAMD17-94-J-4499	ADB258779
DAMD17-94-J-4437	ADB258772
DAMD17-94-J-4437	ADB249591
DAMD17-94-J-4437	ADB233377
DAMD17-94-J-4437	ADB221789
DAMD17-96-1-6092	ADB231798
DAMD17-96-1-6092	ADB239339
DAMD17-96-1-6092	ADB253632
DAMD17-96-1-6092	ADB261420
DAMD17-95-C-5078	ADB232058
DAMD17-95-C-5078	ADB232057
DAMD17-95-C-5078	ADB242387
DAMD17-95-C-5078	ADB253038
DAMD17-95-C-5078	ADB261561
DAMD17-94-J-4433	ADB221274
DAMD17-94-J-4433	ADB236087
DAMD17-94-J-4433	ADB254499
DAMD17-94-J-4413	ADB232293
DAMD17-94-J-4413	ADB240900

Encl